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CLINICAL STUDY

Preliminary study of Huai Qi Huang granules delay the development of primary glomerular diseases in human

Shao-Bin Duan, Peng Pan*, Qian Xu*, Xiejia Li, Na Liu, and Jun Xu

Department of Nephrology, The Second Xiangya Hospital of Central South University, Nephrology Institute of Central South University, Changsha, P.R. China

Abstract

Objective: To evaluate the effects of Huai Qi Huang (HQH) granules on primary glomerulonephritis patients, and to discuss its possible mechanisms. *Method*: Sixteen patients diagnosed with primary glomerular disease between December 2011 and December 2012 were enrolled. Their blood and urine samples were collected at day 0 (the baseline levels), 30, and 90 of receiving HQH granules orally. Levels of creatinine and cystatin C (Cys-C) in serum and urine, and total protein and albumin in urine were measured by automatic biochemical analyzer. Neutrophil gelatinase-associated lipocalin (NGAL) in serum and urine was tested by ELISA; serum malondialdehyde (MDA) was measured by thiobarbituric acid method, the erythrocyte count in urine was calculated under light microscope. *Results*: Serum levels of creatinine, MDA, Cys-C and NGAL at day 30 and 90 were significantly lower than the baseline levels. Urinary levels of Cys-C, NGAL, total protein, albumin and erythrocyte counts were also decreased; level of estimated glomerular filtration (eGFR) was increased. *Conclusion*: HQH granules have certain effect on delaying the development of primary glomerular disease with mild proteinuria and hematuresis in patients. This study may supply a new treatment for primary glomerular diseases.

Keywords

Chronic kidney disease, Huai Qi Huang granules, primary glomerular disease

History

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Introduction

Chronic kidney disease (CKD) has received increasing attention as a leading public health problem around the world. Epidemiology data showed that the prevalence of CKD was 10.8% in China, 13.0% in USA, and 10.2% in Norway.[1–3] The results of a study from China suggested that the primary diseases leading to CKD in these patients mainly include primary glomerular disease (55.2%), hypertensive nephropathy (7.6%) and diabetic nephropathy (6.3%). The primary glomerular disease was the main reason of the hospitalized patients with CKD in China.[4]

Previous studies have found that neutrophil gelatinase-associated lipocalin (NGAL), cystatin C (Cys-C), oxidative stress markers malondialdehyde (MDA), estimated glomerular filtration rate (eGFR), and so on, were sensitive and reliable biomarkers to evaluate CKD progression.[5,6] Glucocorticoids and immunosuppressive agents are the main treatment to alleviate CKD progression, but long-term application causes immune dysfunction and complicated infection, thus affecting recovery. The clinical application of immunomodulatory drugs has thus attracted wide attention.

Huai Qi Huang (HQH) granules are composed of Huaier fermented extract out of mycelium, Chinese wolfberry fruit, and Polygonatum. Pharmacological studies suggested that tramitis robiniophila murr, being rich in polysaccharides, is a biological response modifier and can stimulate many elements of the immune system to enhance immunity, it also has effects of anti-inflammatory, anti-allergic, improving microcirculation, and promoting tissue repair.[7] However, little is known about the effect of HQH granules on the progression of primary glomerular diseases in human. Therefore, the aim of the present study is to determine whether HQH granules could delay the progression of primary glomerular diseases, and to provide a novel treatment of primary glomerular diseases.

Subjects and methods

Subjects and reagents

Sixteen patients (6 male, 10 female, aged 16–67 years, mean age 37.38 ± 13.54 years) who had been diagnosed with primary glomerular disease by renal biopsy from December 2011 to December 2012 were enrolled in this study. The admission criteria were mild proteinuria and hematuria; stage 1–3a CKD, eGFR <120 mL/(min $1.73\,m^2$), ≥ 45 mL/(min $1.73\,m^2$). The exclusion criteria included nephrotic syndrome, hypertension, stage 3b $\sim$ 5 CKD, severe anemia, infection, heart failure, pregnant or lactating women, acute renal failure

*Peng Pan and Qian Xu contributed equally to this work.

Address correspondence to Shao-Bin Duan, Department of Nephrology, The Second Xiangya Hospital, Central South University, Changsha 410011, Hunan, P.R. China. Tel: 86-731-85295000; Fax: 86-731-85533525; E-mail: Duansb528@hotmail.com

on CKD, uncooperative mental patients, etc. Normal control subjects included 15 healthy volunteers with normal renal function from hospital staff (9 males and 6 females, with a mean age of 39.93 ± 10.17 years, ranging from 26 to 60 years). HQH was provided by Gaitianli Pharmaceutical Co. (Qidong, Jianshu Province, China). HQH is primarily composed of trametes robiniophila murr (30%), wolfberry fruit, and polygonatum (70%). NGAL ELISA kit was from Cusabio BioTech Co. Ltd. (Wuhan, China).

Analytical methods

Blood and urine samples were collected from patients at day 0 (the baseline levels), 30, and 90 of receiving HQH granules orally. All samples were centrifuged (3000 rpm, 10 min, 4 °C) to obtain supernatant and stored at −80 °C.

The level of serum Cys-C was measured by colloidal gold particle-enhanced colorimetric immunoassay (Hitachi 7600-20, Tokyo, Japan). The level of NGAL in serum and urine was measured by ELISA Kit. The estimated GFR was calculated by modification of diet in renal disease equation for Chinese patients: $175 \times \mathrm{SCr}^{-1.234} \times \mathrm{age}^{-0.179} \times 0.79$ (if female).[8] The excretion of urinary total protein, albumin, and serum creatinine level were detected by an automatic biochemical analyzer (Hitachi 7170A, Tokyo, Japan). Urinary creatinine was determined by a modified Jaffe's method. All urinary total protein and albumin were adjusted for dilution using the urinary creatinine concentration. The erythrocyte count in urine was calculated under light microscope.

Statistical analysis

The experimental results were expressed as mean values ± SD ($\bar{x} \pm S$). A comparison between normal subjects and patients with HQH granules treatment was performed using the Student t test. Comparison of parameters before and after HQH granules treatment was analyzed using paired t tests. A p value less than 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 17.0 (SPSS, Chicago, IL).

Result

Clinical characteristics of patients

Twenty patients with mild proteinuria and hematuria, stage 1–3a CKD, normal blood pressure who had been diagnosed with primary glomerular disease by renal biopsy were enrolled. Four patients were withdrawn from the study due to pregnancy, diarrhea, and poor compliance. Sixteen subjects (6 males, 10 females, aged 16–67 years, mean age 37.38 ± 13.54 years) completed the survey and examination. Their clinical types included 11 chronic nephritic syndromes, 2 simple hematuria and proteinuria, and 3 IgA nephropathy. Their pathologic types contained five focal segmental glomerulosclerosis, one sclerosis glomerulonephritis, two mesangial proliferative glomerulonephritis, two mild glomerular lesions, one mesangial proliferative sclerotic glomerulonephritis, two segmental mesangial proliferative glomerulonephritis, and three IgA nephropathy.

Effects of HQH granules on level of serum MDA and renal function indexes

As shown in Table 1, compared with the control group, serum creatinine, MDA, Cys-C, NGAL levels were significantly increased and eGFR levels were obviously decreased in patients before treatment ($p < 0.01$). Compared with the basic value before treatment, levels of MDA, Cys-C, NGAL in the serum were significantly lower ($p < 0.01$), the level of serum Cr was lower ($p < 0.05$), and the level of eGFR was significantly increased ($p < 0.05$, $p < 0.01$), respectively, at day 30, 90 after treatment with HQH granules. Compared with day 30 after treatment, the levels of serum NGAL and Cys-C decreased at day 90 ($p < 0.01$), there were no statistically different between level of eGFR and serum creatinine ($p > 0.05$).

Effects of HQH granules on urinary markers of renal injury

As showed in Table 2, the levels of urine NGAL, Cys-C, albumin, total protein and erythrocyte count before treatment were higher than normal subjects obviously ($p < 0.01$). Compared with the basic values before treatment, the levels of urine NGAL, Cys-C, total protein, albumin, excretion and erythrocyte count were significantly decreased, respectively, at day 30, 90 after treatment ($p < 0.01$). Compared with day 30 after treatment, the levels of urinary excretion of NGAL, Cys-C, total protein, albumin, and erythrocyte count at day 90 were significantly lower ($p < 0.01$, $p < 0.01$, $p < 0.05$, $p < 0.01$, respectively).

Table 1. Changes in serum levels of biochemical indexes before and after treatment with Huai Qi Huang granules ($\bar{x} \pm s$, $n = 16$).

	Indexes				
Groups	SMDA (nmol/mL)	SCr (μmol/L)	eGFR (mL/min/1.73 m^2)	SCys-C (mg/L)	SNGAL (μg/L)
Normal subjects	4.08 ± 0.80	81.71 ± 8.76	106.90 ± 4.84	0.68 ± 0.14	44.53 ± 6.32
Treatment subjects	–	–	–	–	–
Baseline	9.56 ± 1.25*	123.12 ± 35.57*	66.88 ± 16.72*	1.15 ± 0.28*	364.10 ± 66.71*
Day 30	8.54 ± 1.41[a]	115.20 ± 32.74[b]	71.95 ± 18.27[b]	1.07 ± 0.28[b]	324.71 ± 61.47[a]
Day 90	6.39 ± 1.18[a,c]	110.97 ± 32.16[a]	74.98 ± 19.10[a]	0.98 ± 0.30[a,d]	277.19 ± 58.60[a,c]

Notes: SMDA, serum malondialdehyde; SCr, serum creatinine; eGFR, estimated glomerular filtration rate; SCys-C, serum cystatin C; SNGAL, serum neutrophil gelatinase-associated lipocalin.
*$p < 0.01$, compared with normal control.
[a]$p < 0.01$, [b]$p < 0.05$; compared with base values.
[c]$p < 0.01$, [d]$p < 0.05$; compared Day 30 after treatment.

Table 2. Effects of HQH granules on urinary markers of renal injury before and after treatment with Huai Qi Huang granules ($\bar{x} \pm s$, $n = 16$).

Groups	Indexes				
	UNGAL (μg/g)	UCys-C (μg/g)	UAlb (mg/g)	UTP (mg/g)	Erythrocyte count
Normal subjects	9.51 ± 2.48	4.74 ± 0.74	23.67 ± 8.10	129.18 ± 32.29	0.30 ± 0.10
Treatment subjects	–	–	–	–	–
Baseline	93.37 ± 39.65*	7.118 ± 2.66*	386.03 ± 124.56*	638.06 ± 219.63*	8.74 ± 3.81*
Day 30	57.43 ± 19.62[a]	4.46 ± 1.51[a]	210.95 ± 84.99[a]	345.24 ± 133.51[a]	5.26 ± 3.15[a]
Day 90	43.85 ± 19.83[a,b]	3.29 ± 0.89[a,b]	166.67 ± 68.36[a,b]	264.91 ± 102.12[a,c]	2.58 ± 1.58[a,b]

Notes: UNGAL, urinary neutrophil gelatinase-associated lipocalin; UCys-C, urinary cystatin C; UAlb, urinary albumin; UTP, urinary total protein.
*$p < 0.01$, compared with normal control subjects.
[a]$p < 0.01$, compared with base values.
[b]$p < 0.01$, [c]$p < 0.05$; compared with Day 30 after treatment.

Discussion

The study demonstrated that HQH granules have certain effect on delaying the development of primary glomerular disease with mild proteinuria and hematuresis in patients. It may supply a new treatment for primary glomerular diseases.

CKD is growing as a global public health problem. The size of the worldwide end-stage renal disease (ESRD) population has been expanding at a rate of 7% per year and is projected to exceed 2 million by 2010.[9,10] The epidemiological surveys showed that prevalence of CKD was 13% in non-institutionalized adults of the United States.[11] The prevalence of proteinuria and hematuria in Japan was 4.7% in men, 3.5% in women and 2.8% in men, 11.0% in women, respectively.[12] The prevalence of CKD and albuminuria in China was 10.8% and 9.4%, respectively. In China, some areas have been carried out CKD epidemiological surveys; in recent years, in a study of Hong Kong including 1201 cases (aged over 20 years) hematuria, proteinuria, urinary abnormalities, hypertension detected rate was 3.2%, 13.8%, 17.4% and 8.7%, respectively.[13] Studies from China indicated that the major cause of ESRD in Chinese adults was glomerulonephritis.[4] The present data have indicated that outcomes of CKD include not only progression to ESRD, but also complications of decreased kidney function and increased risk of cardiovascular disease.[14,15] To improve the disproportionate health care and reduce economic burden in patients with CKD, identifying and treating individuals with early stages of CKD is a proposed option that is increasingly being put forward.[16]

Serum creatinine, although be used routinely in clinical practice and provide useful information, has been found lacking sensitivity of diagnosing early renal damage because of the following limitations. First, creatinine excreted in the urine is not solely a result of glomerular filtration but also renal tubular secretion.[17] Second, after an acute fall in GFR, less creatinine is excreted. Furthermore, serum creatinine concentration may not change until a significant loss of kidney function.

Recent studies have found that NGAL, Cys-C, eGFR, proteinuria, and oxidative stress markers MDA indicators were reliable biomarkers evaluating CKD progression and outcomes.[5] NGAL (25 kDa), a ubiquitous lipocalin iron-carrying protein in promyelocytic cells and late promyelocytic cell differentiation stage synthesis, induce the release of leukocyte granules to eliminate pathogenic microorganisms. Recent studies have found that NGAL protein upregulated in many proximal renal units, especially in regenerated tubular cells after renal ischemic injury. Another evidence suggests that NGAL may even be involved as a mediator of CKD progression, serum NGAL was significantly correlated with the severity of renal damage and the progression of renal function deterioration.[18] Cys-C is a small molecule (13 kDa) that is filtered and metabolized after tubular absorption.[19,20] It is a sensitive biomarker of kidney function in mild-to-moderate kidney disease.[21] In the MMKD study, serum Cys-C predicted CKD progression.[22] Some studies showed that elevated oxidative stress occurs early in CKD, and increases with the progressing of CKD.[23] Oxidative parameter, i.e. MDA was increased in diabetic nephropathy patients. Increased urinary albumin excretion is an established predictor of CKD progression and may reflect both glomerular and tubulointerstitial injury.[24]

Our study showed that levels of serum creatinine, MDA, Cys-C, NGAL, and excretion of urinary Cys-C, NGAL, total protein, and albumin in the patients with CKD were significantly increased and eGFR levels were obviously decreased. It further supported that NGAL, Cys-C, eGFR, proteinuria, and MDA indicators were reliable biomarkers of evaluating CKD progression.

It is well known that proteinuria is one of the key risk factors contributed to CKD outcomes.[25] Curative treatment that will arrest kidney deterioration is desired, innovative agents under investigation for CKD to slow kidney deterioration, such as glucocorticoids, immunosuppressive agents, angiotensin-converting enzyme inhibitors, angiotensin receptor blocker, and others. However, in current treatment, nearly half of the patients progress to unfavorable renal outcomes.[26]

HQH granules, a mixture of Chinese herbs, is primarily composed of trametes robiniophila murr, wolfberry fruit, Polygonatum. Pharmacological experiments indicated that trametes robiniophila murr, being rich in polysaccharides, is a biological response modifier and can stimulate many elements of the immune system to enhance immunity. Animal experiments indicated that HQH granules can obviously reduce proteinuria excretion in rats with Adriamycin nephropathy and IgA nephritis, regulate glomerular nephrin, podocin expression and distribution, and prevent podocyte injury; HQH granules can also increase INF-γ, IL-2 expression, inhibit tumor necrosis factor and interleukin-1 secretion. Meanwhile, tubulointerstitial injury and expression of connective tissue growth factor were obviously reduced by treatment with HQH granules.[27] Clinical studies have shown

that HQH granules can prevent the infection and relapse of primary nephrotic syndrome children, by reducing the inflammatory effects of IL-18 and enhancing the inflammatory inhibitory effects of IL-10.[28] Of noted, the precautions of this granule include that: it should be taken before meals, but should not be taken with any spicy, cold or greasy food; also, it should not be recommended to the patients who have fever or cold. Furthermore, HQH granules contain sucrose so that it should be contraindicated in those patients with diabetes.

The results of this study showed that in patients with primary glomerular disease treated with HQH granules, the levels of urinary excretion of NGAL, Cys-C, protein, levels of serum MDA and SCr; eGFR level obviously increased. In addition, the study also found that HQH can alleviate hematuresis and reduce erythrocyte count, but the mechanism is not yet clear. Our findings suggested that HQH can significantly reduce proteinuria, hematuria, and slow CKD progression.

In summary, HQH particles may play an important role in reducing mild proteinuria, hematuria, and slowing CKD progression in patients with primary glomerular diseases. The mechanism may be related to stimulating many elements of the immune system to enhance immunity, effects of anti-inflammatory, anti-allergic, improving microcirculation, and promoting tissue repair. Further well-designed random control trials, including a large number of patients from multi-center, are still necessary to evaluate the role of HQH granules on primary glomerular diseases, especially with the results of long-term follow-up study.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article. This work was supported by grant from the Scientific Foundation of Hunan Province, China (2010FJ6008, S2013F1022).

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